Clinical Features and Molecular Genetics:

Early infantile epileptic encephalopathy (EIEE), also known as Ohtahara syndrome, is a severe form of epilepsy characterized by frequent tonic spasms with onset in the first months of life. EEG reveals suppression-burst patterns, characterized by high-voltage bursts alternating with almost flat suppression phases. Seizures are medically intractable with evolution to West syndrome at 3-6 months of age and then Lennox-Gastaut syndrome at 1-3 years of age. EIEE represents approximately 1% of all epilepsies occurring in children less than 15 years of age (1). Patients have severe developmental delay and poor prognosis. The diagnostic workup of EIEEs remains challenging because of frequent difficulties in defining etiologies. Acquired structural abnormalities like hypoxic-ischemic insults and isolated cortical malformations, which represent the most common causes of epileptic encephalopathy in infancy should be excluded first (2).

Our EIEE Panel includes sequence analysis of all 30 genes listed below and deletion/duplication analysis of all 21 genes listed in bold.

<table>
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<tr>
<th>Early Infantile Epileptic Encephalopathy Panel</th>
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<tr>
<td>ALDH7A1</td>
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<td>ARFGEF2</td>
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Gene | Inheritance | Clinical Features and Molecular Pathology |
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<tr>
<td>ALDH7A1 [OMIM#107323]</td>
<td>AR</td>
<td>Mutations in ALDH7A1 are associated with pyridoxine-dependent epilepsy (PDE), which is characterized by EIEE that is resistant to antiepileptic drugs but responsive to large doses of pyridoxine (vitamin B6) (3).</td>
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<tr>
<td>ARFGEF2 [OMIM#605371]</td>
<td>AR</td>
<td>Homozygous splice-site mutations in the ubiquitously expressed ARFGEF2 gene were identified in a consanguineous Pakistani kindred with multiple children who presented with West syndrome, evolving to Lennox–Gastaut syndrome with severe intellectual disability, microcephaly and associated with diffuse periventricular heterotopia (4).</td>
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<tr>
<td>ARHGEF9 [OMIM#300607]</td>
<td>XL</td>
<td>Shimojima et al. (2011) identified a nonsense mutation in ARHGEF9 in one out of 23 males with severe intellectual disability and epilepsy (5). A missense mutation in ARHGEF9 in a male with severe intellectual disability, hyperekplexia (excessive startle) and refractory infantile-onset epilepsy has also previously been described (6).</td>
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<tr>
<td>ARHGEF15 [OMIM#608504]</td>
<td>AD</td>
<td>In a group of children with difficult to control epilepsy and developmental delay, Veeramah et al. (2013) found a single patient with a de novo mutation in ARHGEF15 which codes for Ephexin5 (7). The degradation of Ephexin5 promotes synapse development and is mediated by the protein coded for by UBE3A (8).</td>
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<tr>
<td>ARX [OMIM#300382]</td>
<td>XL</td>
<td>Up to 10% of males with a clinical diagnosis of EIEE or West syndrome/cryptogenic infantile spasms may have mutations in the ARX gene (1, 9). Carrier females of ARX mutations can be asymptomatic (10).</td>
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<tr>
<td>CDKL5 [OMIM#300203]</td>
<td>XL</td>
<td>Archer et al. (2006) identified CDKL5 mutations in 7/42 (17%) of females with severe mental retardation and seizures in the first 6 months of life (11). The most common feature found in patients reported to date with CDKL5 mutations is the early onset of seizures. CDKL5 mutations have been reported in more female than male patients, however, Elia, et al (2008) reported CDKL5 mutations in 3/8 boys with severe mental retardation and early-onset seizures (12).</td>
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<tr>
<td>Gene</td>
<td>Inheritance</td>
<td>Description</td>
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<td>CHD2</td>
<td>AD</td>
<td>In a cohort of 500 patients with epileptic encephalopathy, 1.2% were found to have de novo mutations in CHD2 (13). Reported features include: Dravet syndrome (14), myoclonic seizures, absence seizures, photosensitivity, and moderate to severe intellectual disability (13). Mutations leading to protein truncation, as well as missense mutations have been reported.</td>
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<tr>
<td>CLCN4</td>
<td>XL</td>
<td>In a group of children with difficult to control epilepsy and developmental delay, Veeramah et al (2013) found a single patient with a hemizygous de novo missense mutation in CLCN4 (7). In addition to epilepsy, this child had microcephaly, hypotonia, myotonia, and intellectual disability.</td>
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<tr>
<td>EEF1A2</td>
<td>AD</td>
<td>In 2012, de Ligt et al. identified a de novo missense mutation in a patient with a history of neonatal hypotonia and seizures at 4 months and intellectual disability, autistic features, and aggressive behavior (15). Subsequently, in a group of children with difficult to control epilepsy and developmental delay, Veeramah et al (2013) found a single patient with a de novo missense mutation in EEF1A2 (7).</td>
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<tr>
<td>EFHC1</td>
<td>AD/AR</td>
<td>Berger et al. (2012) described a family with primary intractable epilepsy in infancy with a homozygous missense mutation in EFHC1 (16). The affected children died at 18-36 months of age. Heterozygous mutations in EFHC1 have been associated with increased susceptibility to juvenile myoclonic epilepsy (17).</td>
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<tr>
<td>GNAO1</td>
<td>AD</td>
<td>Nakamura et al. (2013) identified de novo mutations in four individuals with EIEE, three of whom were described as having Ohtahara syndrome, and two of whom had involuntary movements (18). One of the patients had somatic mosaicism, with only one third to half of various cell types harboring the mutation. Missense mutations and a small in-frame deletion were reported.</td>
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<tr>
<td>GRIN2A</td>
<td>AD</td>
<td>Endele et al. (2010) found heterozygous GRIN2A mutations in two out of 127 individuals with a history of idiopathic epilepsy and/or abnormal EEG findings and a variable degree of intellectual disability (19). The most consistent clinical feature seen in individuals with GRIN2A mutations is epilepsy, which varies in severity between individuals. The most severe cases are associated with EIEE (19).</td>
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<tr>
<td>KCNH5</td>
<td>AD</td>
<td>In a group of children with difficult to control epilepsy and developmental delay, Veeramah et al. (2013) found a single patient, whose presentation was consistent with West syndrome, with a de novo heterozygous mutation in KCNH5 (7).</td>
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<tr>
<td>KCNQ2</td>
<td>AD</td>
<td>Weckhuysen et al. (2012) identified heterozygous KCNQ2 mutations in 8 out of 80 patients with EIEE, 6 of which were confirmed to be de novo. Mutations in KCNQ2 can also be associated with other phenotypes including benign familial neonatal seizures (BFNS), which is an autosomal dominant seizure disorder typically associated with a good prognosis (20). Parental mosaicism has been described in one family with a mutation in KCNQ2 (20, 21).</td>
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<tr>
<td>KCNT1</td>
<td>AD</td>
<td>Barcia et al. (2012) identified de novo heterozygous gain-of-function mutations in KCNT1 in six patients with a subtype of EIEE known as malignant migrating partial seizures of infancy (22). Missense mutations in KCNT1 have also been reported in families with autosomal dominant nocturnal frontal lobe epilepsy-5 (23).</td>
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<tr>
<td>PCDH19</td>
<td>XL</td>
<td>Mutations in the PCDH19 gene have been associated with EIEE. Marini et al. (2010) identified 13 different mutations in the PCDH19 gene in 13 (11%) of 117 female patients with febrile seizures and a wide spectrum of epilepsy phenotypes (24). PCDH19 mutations are X-linked, with the phenotype being restricted to females. Males with hemizygous mutations are apparently unaffected with normal cognitive functions. This unusual mode of inheritance is likely to be due to cellular interference, a mechanism assuming that only the co-existence of PCDH19 positive and negative cells, as a result of random X inactivation in females, is pathogenic (11).</td>
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<tr>
<td>PLCB1</td>
<td>AR</td>
<td>Kurian et al. (2010) identified a homozygous deletion of the promoter element and exons 1-3 of the PLCB1 gene in a child with EIEE from a consanguineous family of Bangladeshi descent.</td>
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<tr>
<td>PNKP</td>
<td>AR</td>
<td>Mutations in the PNKP gene are associated with early-onset intractable epilepsy, microcephaly, developmental delay and behavioral abnormalities (25). Missense and frameshift mutations identified in PNKP have been associated with severe encephalopathy, whereas an intronic deletion identified in one individual, that was predicted to disrupt proper mRNA splicing, was associated with a milder phenotype (25, 26). The PNKP protein is involved in DNA repair of both double and single-stranded breaks, however at this time no features typically associated with DNA repair defects, such as cancer predisposition or immunological abnormalities have been reported in affected individuals (25).</td>
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</table>
| PNPO     | AR          | Mills et al (2005) found homozygous mutations in PNPO in 3 individuals with early infantile epileptic encephalopathy and biochemical changes in the CSF, indicative of reduced activity of aromatic L-amino acid decarboxylase (AADC) (27). This was found to
be due to deficiency of pyridoxal phosphate (PLP), which is a co-factor of AADC and is synthesized by the enzyme encoded for by the PNPO gene. Of the 3 affected children, only one was treated with PLP and survived the neonatal period, however he continued to have symptoms such as seizures, severe developmental delays and dystonic spasms (27).

**POLG**
- **[OMIM#174763]**
  - **AR**
  - In a study of 213 children with early or juvenile onset nonsyndromic intractable epilepsy, Uusimaa et al. (2013) identified 5 (2.3%) with compound heterozygous or homozygous mutations in the POLG gene (28). The majority of patients had elevated cerebrospinal fluid lactate. A proportion of affected individuals may develop liver failure, particularly if their seizures are being treated with sodium valproate (28).

**SCN1A**
- **[OMIM#607208]**
  - **AD**
  - Mutations in the SCN1A gene can cause EIEE6, which is more commonly known as Dravet syndrome. EIEE6 is characterized by onset of seizures in the first year of life, often triggered by fever, photostimulation, or modest hyperthermia, which usually evolve to include myoclonic seizures over time (29). Mutations in SCN1A associated with EIEE6 are typically de novo (1). Of those with a clinical diagnosis of EIEE6, 85% have a mutation in **SCN1A** (29).

**SCN2A**
- **[OMIM#182390]**
  - **AD**
  - Ogiwara et al. (2009) identified 2 de novo mutations in the SCN2A gene in a cohort of 116 patients with intractable childhood epilepsies (30). Mutations in the SCN2A gene can also cause benign familial neonatal seizures (BFNS).

**SCN8A**
- **[OMIM#600702]**
  - **AD**
  - Veeramah et al. (2012) identified a de novo mutation in **SCN8A** in a female with EIEE (31). Symptoms started at 6 months of age with refractory generalized seizures, and the patient died suddenly at age 15 years (31).

**SLC25A22**
- **[OMIM#609302]**
  - **AR**
  - Homozygous mutations in **SLC25A22** have been described in case reports of consanguineous families with EIEE (30, 32).

**SLC2A1**
- **[OMIM#606777]**
  - **AD**
  - Glucose transporter-1 (GLUT1) deficiency syndrome is caused by heterozygous mutations in the SLC2A1 gene, which lead to impaired glucose transport in the brain. The classic GLUT-1 deficiency syndrome presentation is drug-resistant infantile-onset seizures, developmental delay, acquired microcephaly, hypotonia, spasticity, ataxia and dystonia (32). Seizures are typically refractory and worsen during periods of fasting (33). The majority of reported cases are due to de novo mutations (34).

**SPTAN1**
- **[OMIM#182810]**
  - **AD**
  - Saitsu et al. (2010) identified de-novo heterozygous mutations in 2 unrelated Japanese patients with EIEE (35).

**ST3GAL3**
- **[OMIM#606494]**
  - **AR**
  - A homozygous mutation in **ST3GAL3** was identified in a consanguineous Palestinian family with four individuals affected by severe early infantile epileptic encephalopathy (36). Mutations in **ST3GAL3** have also been described in patients with mild to moderate non-syndromic intellectual disability (36).

**ST3GAL5**
- **[OMIM#604402]**
  - **AR**
  - Mutations in **ST3GAL5** are associated with infantile onset of refractory and recurrent seizures, associated with profoundly delayed psychomotor development, abnormal movements, and vision loss (37). A founder mutation is present in the Amish community (37). A homozygous mutation was also found in an affected child of French ancestry.

**STXBP1**
- **[OMIM#602926]**
  - **AD**
  - Sequencing of **STXBP1** detected mutations in 4 out of 106 patients with EIEE (38). Earlier reports identified 4 heterozygous missense mutations in 13 patients with EIEE (39). Parental mosaicism has been described in one family with a mutation in **STXBP1** (20, 21).

**SZT2**
- **[OMIM#615463]**
  - **AR**
  - In 2 unrelated patients with EIEE, Basel-Vanagaite et al. (2013) identified biallelic truncating mutations in the **SZT2** gene (40). The phenotype was characterized by lack of psychomotor development apparent from birth, dysmorphic facial features, early onset of refractory seizures, and thick corpus callosum and persistent cavum septum pellucidum on brain imaging.

**Test methods:**
Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All novel and/or potentially pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp.

Deletion/duplication analysis of the panel genes is performed by oligonucleotide array-CGH. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by array-CGH. Array-CGH will not detect low-level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of this assay may be reduced when DNA is extracted by an outside laboratory.
Our Infantile and Childhood Epilepsy Epilepsy Panel is also available (see website). In addition, sequencing and deletion duplication analysis of individual genes is offered separately for several of the genes on the EIEE panel, including ARX, CDKL5, PCDH19, PNKP, STXBP1, SLC25A22 and SPTAN1. Please see our website for more details regarding these other test options.

**EIEE Next Generation Panel (sequence analysis of 30 genes and deletion/duplication analysis of 21 genes)**

- Sample specifications: 3 to 10 cc of blood in a purple top (EDTA) tube
- Cost: $4,500
- CPT codes: 81479
- Turn-around time: 8 weeks

**Note:** We cannot bill insurance for the EIEE panel

**Note:** The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

**Results:**

Results, along with an interpretive report, are faxed to the referring physician as soon as they are completed. One report will be issued for the entire EIEE panel. All abnormal results are reported by telephone.

**References:**


Committed to CUSTOMIZED DIAGNOSTICS, TRANSLATIONAL RESEARCH & YOUR PATIENTS’ NEEDS